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Primary Structure of Mouse Actin-Related Protein 1 (Arp1) and Its Tissue Expression

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ABSTRACT—Different types of actin-related proteins which constitute an actin-superfamily together with conventional actin have recently been described (Mullins *et al.*, 1996). Among them, Arp1 exhibits the highest homology with conventional actin. With the aim of clarifying the cellular function of Arp1 in mammalian cells, we cloned the cDNA encoding mouse α-Arp1, one of the variants of Arp1, from a mouse diaphragm cDNA library; two types of α-Arp1 cDNAs, which are probably generated by alternative RNA splicing from a single gene, were obtained and the entire sequences were determined. They differed only in the presence or absence of an insertion of 1.3 kb in the 3'-non-coding region but shared a common open reading frame. The deduced amino acid sequence was identical with that of human α-Arp1. Northern blot analysis showed that the α-Arp1 mRNA corresponding to the longer cDNA is transcribed not only in various non-muscle tissues but also in muscle tissues, while the transcript corresponding to the shorter one becomes expressed only in skeletal muscle as development progresses. It is suggested that α-Arp1 may play some role in muscle, as judged by the significant level of its expression.

INTRODUCTION

Actin is an abundant and ubiquitous protein which is widely distributed in eukaryotes and is involved in a variety of cellular functions, such as muscle contraction, cell motility, maintenance of cell shape, cell migration and cytokinesis. Multiple actin isoforms, six in higher vertebrates, have been distinguished (Vandekerckhove and Weber, 1978). They are highly conserved in the primary sequence (Herman, 1993) and exhibit similar functional properties in *in vitro* biochemical assays (Rubenstein, 1990). However, the actin isoforms exhibit distinct expression patterns and differential localization in the cytoplasm of a variety of cells (Herman, 1993; Arx *et al.*, 1995; Hayakawa *et al.*, 1996), suggesting that they may play somewhat different roles in the cytoplasm. Such difference may be partly due to their intrinsic nature and differential interaction with actin-binding proteins. Recently, novel genes encoding for the proteins (actin-related proteins; Arps) related in primary sequence to conventional actins were reported (Mullins *et al.*, 1996; Frankel and Mooseker, 1996). They are regarded as constituting an actin-superfamily together with conventional actin isoforms and HSC70. These actin-related proteins have been classified into Arp1, Arp2, Arp3, Arp4, Arp5 and Arp6, and several variants have been described in each class (Mullins *et al.*, 1996; Frankel and Mooseker, 1996). The sequence homology to conventional actin has described as about 53-54% (Arp1), 47% (Arp2), 35-40% (Arp3), respectively (Schroer *et al.*, 1994; Mullins *et al.*, 1996; Frankel and Mooseker, 1996). Among these actin related proteins, Arp1 (also called centractin or actin RPV) is a class of protein most related to conventional actin as judged by the primary sequence (Mullins *et al.*, 1996; Frankel and Mooseker, 1996), the tertiary structure (Mullins *et al.*, 1996) and the functional properties (Schroer 1994; Schroer *et al.*, 1996). Recently, it was suggested that Arp1 copolymerizes with conventional actin *in vitro* (Melki *et al.*, 1993), although Arp1 was originally detected as a major component in the dynactin complex, a cytoplasmic dynein activator (Gill *et al.*, 1991; Schafer *et al.*, 1994). Therefore, it seems likely that Arp1 modulates actin assembly and/or actin filament organization, although very little is known as to the details.

We have been concentrating our studies on actin dynamics during muscle differentiation for some time (Obinata, 1993). During muscle development, actin isoforms switch from non-muscle (β, γ) types to sarcomeric (α) types. In addition, dynamic redistribution of actin occurs as muscle differentiation progresses. In developing muscle cells, a considerable amount of actin is pooled as monomer in the cytoplasm (Shimizu and Obinata, 1986) and actin filaments are detectable mostly in the cortical region of the cells at the early stage of myofibrillogenesis (Antin *et al.*, 1986). As myofibrillogenesis progresses, the cortical actin filaments may be redistributed into thin filaments of myofibrils and, on the other hand, newly

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synthesized G-actin is assembled into the thin filaments (Obinata, 1993). Several actin-binding proteins involved in the process of actin reorganization have been characterized. Since the expression of Arp1 in muscle tissues has been briefly described (Clark *et al.*, 1994), it is assumed that Arp1 may play some role in the process of actin filament organization in developing muscle in addition to the actin-binding proteins.

In this study, as the first step toward clarifying the contribution of Arp1 to the actin dynamics, we cloned and determined the entire sequences of mouse α-Arp1 cDNAs. We observed that while α-Arp1 is most abundant in brain, it is also expressed in various other mouse tissues including muscle, and that α-Arp1 mRNA with a much shorter 3'-non-coding sequence is generated specifically in skeletal muscle.

MATERIALS AND METHODS

PCR amplification of mouse α-Arp1 cDNA fragment

All DNA manipulations in this study were performed according to standard procedures (Sambrook *et al.*, 1989) unless otherwise noted. The primers for amplification of α-Arp1 cDNA were based on the sequence from canine α-centractin cDNA (Clark and Meyer, 1992). Two oligonucleotides were synthesized on an Applied Biosystems 391 DNA synthesizer (PCR-MATE™). The forward oligonucleotide was 5'-CCATGGAATCCTACGATG-3' containing the initiation codon and the *Nco*I site (underlined), and the reverse oligonucleotide was 5'-ATTAGAAGGTTTTCCTGTGGATG containing the nonsense codon (underlined). The Uni-Zap XR® mouse diaphragm cDNA library (Toyobo 937303) was used as a template. The reactions were carried out at 95°C for 1 min, 42°C for 2 min and 72°C for 2 min for 30 cycles. The amplified products were blunted by a Klenow fragment and cloned into the *Eco*RV site of pBluescript II KS+.

cDNA cloning and sequencing

The PCR product (pPCR#2) of 1.1 kb was used as a DNA probe to screen the Uni-Zap XR® mouse diaphragm cDNA library. Seven positive clones were identified and rescued as phagemid (Stratagene). Double stranded DNA sequencing was performed by the dideoxy termination method (Sanger *et al.*, 1977) using BcaBEST sequencing kit (Takara). The sequence data were analyzed on GENETYX-Mac software (SDC).

Northern blot analysis

Total RNA was prepared from mouse tissues and cultured muscle cells by the rapid one-step method (Chomczynski and Sacchi, 1987). 20 μg of RNA was applied for each lane and was separated on 0.8% agarose-formamide gel and transferred to nitrocellulose filters, which were then cross-linked by UV cross-linker (Stratagene). Hybridization was carried out by the procedure of Thomas (1980), with ^{32}P-labeled probes by the method of Feinberg and Vogelstein (1983). The filters were finally washed in 0.2 × SSC (30 mM NaCl, 5 mM trisodium citrate) containing 0.1% SDS at 55°C. The washed filters were exposed to a BAS imaging plate (Fuji film).

Southern blot analysis

Chromosomal DNA from mouse liver was prepared as described by Blin and Stafford (1976), and 10 μg of DNA was digested by *Eco*RI, *Bam*HI, *Hin*dIII, *Kpn*I, and *Xba*I, electrophoresed on 0.7% agarose gel, and then transferred to nitrocellulose filters which were then cross-linked by a UV cross-linker. Hybridization was performed using a fragment of the cDNA coding region of mouse α-Arp1 prepared from the *Bam*HI to *Eco*RV site (see Fig. 1) as a probe, as described under "Northern blot analysis". The filters were finally washed in 0.2 × SSC containing 0.1% SDS at 65°C.

Cell culture

Sol8 cells (Mulle *et al.*, 1988) were grown in Dulbecco's modified Eagle's medium (DMEM), supplemented with 20% fetal bovine serum and 15 μg/ml kanamycin. Cells were grown on culture dishes and differentiated in DMEM supplemented with 5% horse serum and 15 μg/ml kanamycin.

RESULTS

Cloning and sequencing analysis of mouse α-Arp1

In order to clarify the expression and cellular function of Arp1, we cloned cDNAs encoding mouse α-Arp1. Initially, we obtained a cDNA fragment of mouse α-Arp1 by PCR methods. Based on the information available from the canine α-Arp1 (α-centractin) sequence (Clark and Meyer, 1992), we created PCR primers (see MATERIALS AND METHODS) and successfully cloned α-Arp1 sequences using a mouse diaphragm cDNA library as a template. One of the clones (pPCR#2, Fig. 1) appeared to encode α-Arp1, since the

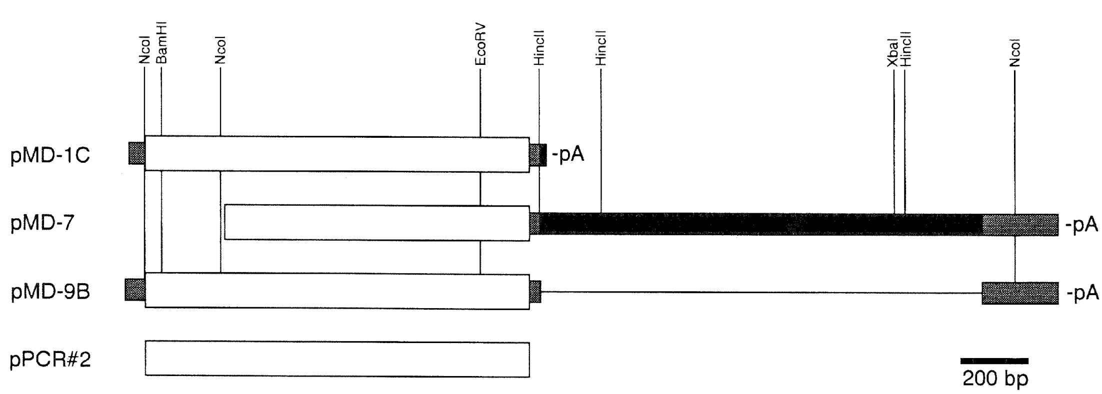

Fig. 1. Restriction map of the cDNAs encoding mouse α-Arp1. The cDNA clones encoding mouse α-Arp1 (pPCR#2, pMD-1C, -7 and -9B) are schematically shown. The 5'-end is on the left. Open boxes indicate the coding regions, and the non-coding regions are indicated by shadowed and closed boxes. The closed boxes further indicate an insertion sequence detected in pMD-1C and -7. pA, poly (A) tail; bp, base pairs.

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deduced amino acid sequence was detected in canine α-Arp1 (data not shown).

To obtain the cDNA containing the complete sequence of mouse α-Arp1, we further screened the mouse diaphragm cDNA library using ^{32}P-labeled pPCR#2 as a probe. Several clones were isolated, and the sequences of the clones of 1.2 kb, 2.4 kb and 1.45 kb, named pMD-1C, pMD-7 and pMD-9B, respectively, were determined (see Fig. 1). The clone pMD-9B contained a complete 1,128 bp open reading frame encoding 376 amino acid residues (Fig. 2). The isoelectric point of the protein was deduced as 6.19 from the sequence. A poly (A) tail was detected in pMD-9B which followed 16 bp downstream from the polyadenylation signal sequence AATAAA (position 1419-1424) (Fig. 2). The clone pMD-7 shared the same sequence with pMD-9B except that pMD-7 lacked the 5'-side sequence up to the position 280 of pMD-

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                                            ▼
                                GGCGTTTGCTGCGGACCGGGCTGGCAGTTCCTTCCTCGGAGGGAAAGATTCCTCTGCC   58
ATGGAGTCCTACGATGTGATCGCCAACCAGCCTGTTGTGATCGACAACGGATCCGGTGTGATTAAAGCTGGCTTTGCTGGTGATCAGATC  148
M  E  S  Y  D  V  I  A  N  Q  P  V  V  I  D  N  G  S  G  V  I  K  A  G  F  A  G  D  Q  I    30
CCCAAATACTGCTTTCCAAACTATGTGGGCAGACCCAAACATGTTCGTGTCATGGCAGGAGCCCTGGAAGGTGACATCTTCATTGGCCCC  238
P  K  Y  C  F  P  N  Y  V  G  R  P  K  H  V  R  V  M  A  G  A  L  E  G  D  I  F  I  G  P    60

                                             ▽
AAAGCGGAGGAGCACCGGGGGCTGCTGTCAATCCGTTACCCCATGGAACATGGCATCGTCAAGGACTGGAACGACATGGAACGCATCTGG  328
K  A  E  E  H  R  G  L  L  S  I  R  Y  P  M  E  H  G  I  V  K  D  W  N  D  M  E  R  I  W    90
CAATACGTCTACTCTAAGGACCAGCTGCAGACTTTCTCAGAGGAGCATCCTGTGCTCCTAACGGAGGCACCTTTAAACCCTCGGAAAAAC  418
Q  Y  V  Y  S  K  D  Q  L  Q  T  F  S  E  E  H  P  V  L  L  T  E  A  P  L  N  P  R  K  N   120
CGGGAGCGAGCTGCAGAAGTCTTCTTCGAGACCTTCAATGTGCCGGCCCTCTTCATCTCCATGCAAGCCGTGCTGAGCCTTTATGCCACG  508
R  E  R  A  A  E  V  F  F  E  T  F  N  V  P  A  L  F  I  S  M  Q  A  V  L  S  L  Y  A  T   150
GGCAGAACCACAGGCGTGGTGTTGGATTCTGGGGATGGTGTCACCCATGCAGTCCCGATTTATGAAGGCTTCGCCATGCCTCACTCCATC  598
G  R  T  T  G  V  V  L  D  S  G  D  G  V  T  H  A  V  P  I  Y  E  G  F  A  M  P  H  S  I   180
ATGCGCATCGACATCGCTGGCCGGGATGTCTCACGCTTCCTCCGCCTCTACCTACGGAAGGAGGGCTATGATTTCCACTCCTCCTCTGAG  688
M  R  I  D  I  A  G  R  D  V  S  R  F  L  R  L  Y  L  R  K  E  G  Y  D  F  H  S  S  S  E   210
TTTGAGATTGTCAAGGCCATAAAGGAAAGAGCTTGCTACCTGTCCATAAACCCCCAGAAGGATGAGACACTGGAGACAGAGAAGGCTCAG  778
F  E  I  V  K  A  I  K  E  R  A  C  Y  L  S  I  N  P  Q  K  D  E  T  L  E  T  E  K  A  Q   240
TACTACCTGCCCGATGGCAGCACCATTGAGATTGGCCCTTCCCGGTTCCGGGCCCCTGAGCTGCTGTTCAGGCCGGACTTGATTGGCGAG  868
Y  Y  L  P  D  G  S  T  I  E  I  G  P  S  R  F  R  A  P  E  L  L  F  R  P  D  L  I  G  E   270
GAGAGTGAAGGCATCCACGAGGTGCTGGTGTTTGCTATCCAGAAGTCAGACATGGATTTACGGCGTACACTGTTTTCCAACATTGTCCTC  958
E  S  E  G  I  H  E  V  L  V  F  A  I  Q  K  S  D  M  D  L  R  R  T  L  F  S  N  I  V  L   300
TCAGGAGGTTCTACCCTGTTCAAAGGTTTTGGAGACAGGCTACTGAGTGAAGTGAAGAAACTAGCTCCAAAAGACGTGAAGATCAGGATA 1048
S  G  G  S  T  L  F  K  G  F  G  D  R  L  L  S  E  V  K  K  L  A  P  K  D  V  K  I  R  I   330
TCTGCACCCCAGGAGAGACTGTATTCCACATGGATTGGAGGTTCTATCCTTGCTTCCCTGGACACCTTTAAGAAGATGTGGGTCTCTAAG 1138
S  A  P  Q  E  R  L  Y  S  T  W  I  G  G  S  I  L  A  S  L  D  T  F  K  K  M  W  V  S  K   360
AAAGAATATGAAGAAGACGGTGCCCGATCCATCCACAGGAAAACCTTCTAACGTTGCAACATCACCACCTCTCTCTGAAGTTAACTCCAC 1220
K  E  Y  E  E  D  G  A  R  S  I  H  R  K  T  F  *                                          376
TTTAAAACTCGCTTTCTTGAGTTGGAGTGTTTGAGAGGAACTGCCTGTGTGTGTGAATGAGTGTGTGGGTATGTATGAGTGTGTGCGACT
ATCGAGTGCCGTGTGGCCCAGAGATCCTGGGCCCAGAGAGGACATTGAACTACCCACGATGGTGACGGCCTGAGGCCTGGGGTTGACTAC
TAACTGACCCCTTACAGGAAGAGCTCTGGCAGAGGCCCACTCCTTTCTCAGCCAGGCCTTTGTCTGGCTGTGCGGAACTATGGTTACTAC
CATAGAGAGACCTTGCTGCTGCCGATCTAGGCTGGGCTGGCCCCACCCCACCCCTCCCCACCCGAGTGCCCTGAGGCCGGAGGCAGCTGC
TGTCTCCCCTGCTCATCCTTCTGTGCTGTCCATGCCTCTGGGGGGCGGGGTTAAGTTCTGCCCGGTTTTGTTTCAGTTTGGGTTTGGGAG
ACTGAAAAAGCAGTCTGGCCGTGGTTAGAGACTGGGTTCAAGAGAGAGCAGGGTACACTCGTGTCCACCTGGGAACCAGGTAGGGATTAG
GACCACACTGGGCATTTGAGGACAGGAGATAGCTTCAGGGTTAATGTACAAGCCCCAGCCACATCCAGGGGCCCTGAGACACTGTAAGGA(1303)
CTGTGGGCTGCAGGTCCTGCTGTTGGCTTCTCCTACGTCCTGGTTTCGATGCCATCATCACGGTTTTATTTATTGACGTGTTTGTTCAGT
AAAGGGACTACAGAGAGGGCTCGCTGTCTCCTACCCACCGGTCACCTTGCTTACACTAATGTTTACAGCACCTAAGTTTCCCATAGCATC
CAGGCCTCTGTCATTCCCTGCTGATGGAGAACTGACAGTATTCATGGGCCTCAGGACCCTGTGATTGGAGGCTGCACAGGAAGGCACCCC
AGCCAGCCTCTTCCTTTCTCCTAACCCCACTTGCCCACTGCCCTGGAACCAACAGGCTGGTTGTGGTTGGATTTTCTAAGACAGAAAGTA
GCATCTCTCTGTGGCAGAGGCCCCTTGCGAGGGAGGTGTGTTGGAGATCTAGACCCTTTAAAACAAAGCAAGCTGCAGTTAACCATGTTG
CCTTTTGCCACTCTAATTGACCAGTGGACTAAATGGCAGGGAGGCCCCTTCTGGGTTGGTCAGATTGGAGAGAAGGGGCTGACCAAAGTG
CCAAGACCTAATGGGTGCCAGCCTTGCTCTCAGAAGTGGTCCAAGGGCCTTTGCGCCTGGTACAAGCTAGGGTGAGCCTGTCTTCACCGG
CACCCTCACCCGTGACACCAAGTCTCGCCTCAGTTCCAGATTCCAGACAGTATTCTTTCTCCGCCATCCTATGACCAAAGGTCCCTGCCA 1275
GATGTAAAGCGAAGCTGGTGTGTGTGGAATCAGTGTGGCAACCCATGGACTGCAGTGCGCATAACCAGCTCACCTCTTAGCCCAAGCCTG 1365
TCCCTTGCACAGCCTCGCGAAGCCACTTTGCCTGGTGGGGCCCAGTGTACTTAAATAAAGTCGTTCCAATAGGT-(pA)          1439
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Fig. 2. The nucleotide sequence of mouse α-Arp1 and the deduced amino acid sequence. The sequence of pMD-9B was combined with the insertion sequence (denoted by black boxes) observed in pMD-7. The 5'-ends of pMD-1C and pMD-7 correspond to position 12 (marked by closed triangle) and 281 (marked by open triangle), respectively. The putative polyadenylation signal sequence is underlined. Shadowed and black-boxed regions correspond to those in Fig.1.

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9B, but interestingly, it contained a long insertion sequence of 1,303 bp in the 3'-non-coding region, namely between positions 1220 and 1221 of pMD-9B (Fig. 1 and Fig. 2). It is very likely that the cDNAs with or without the insertion in the 3'-non-coding region are generated from a single gene by alternative RNA splicing, because the entire sequences of the cDNAs except the insertion sequence were completely the same. The shortest cDNA clone pMD-1C shared the complete open reading frame with pMD-9B and had only a short 3'-non-coding sequence (37 bp) that was detectable in pMD-7 and a poly (A) tail. We assume that pMD-1C and pMD-7 were derived from the same transcript, but the part of the 3'-non-coding sequence was deleted artificially by unknown reasons during the cloning process of the former, which lacked a polyadenylation signal, and the mRNA which corresponding to this cDNA was not detected by Northern blotting (Fig. 3). The amino acid sequences deduced from pMD-9B and pMD-1C (Fig. 2) were completely identical with canine α-Arp1 (Clark and Meyer, 1992), human α-Arp1 (Lees-Miller *et al.*, 1992) and *Drosophila* Arp1 (Fyrberg *et al.*, 1994).

Distribution of mRNAs encoding α-Arp1 in mouse tissues and cultured cells

Total cytoplasmic RNA isolated from several adult mouse tissues was examined by Northern blotting with the entire cDNA encoding α-Arp1 (pMD-9B) as a probe. As shown in Fig. 3, mRNA of about 2.8 kb was detected in all the tissues examined. The amount was most abundant in brain. There was an additional band of 1.5 kb detectable only in skeletal muscle. These two types of mRNAs differing in size probably correspond to the cloned cDNAs with or without the insertion sequence.

We further examined the expression of α-Arp1 during muscle development using sol8 myogenic cells, a mouse skeletal muscle cell line. As shown in Fig. 4, the 2.8 kb message for α-Arp1 was detected in both myoblasts and myotubes, but the 1.5 kb message was detected only in the myotubes cultured for a longer period. The appearance of α-sarcomeric actin apparently preceded the generation of the 1.5 kb message of α-Arp1 (Fig.4). These observations then suggest that the generation of the mRNA of 1.5 kb is a phenomenon related to muscle maturation.

Southern blot analysis of mouse genomic DNA

Southern blot analysis of mouse genomic DNA was carried out using a fragment of about 950 bp of the α-Arp1 cDNA, which constitutes approximately four-fifths of the coding region. This region of the cDNA was selected as a probe since it seemed to be the most conserved region in the Arp1 sequences. As shown in Fig. 5, the α-Arp1 cDNA strongly hybridized with a major single band in the genomic DNA digest by each restriction enzyme but only weakly with an additional band in the digests by *Kpn*I and *Xba*I. As judged from these results, it is very likely that there is only a single gene for mouse α-Arp1, and that the two transcripts for α-Arp1 are generated from the same gene by alternative splicing.

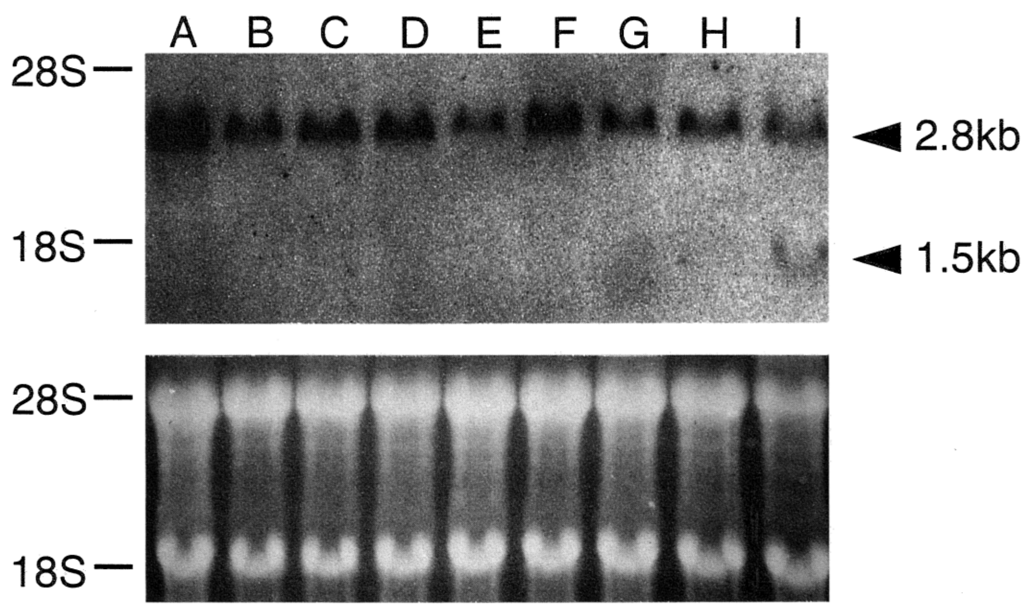

Fig. 3. Distribution of mRNA for α-Arp1 in mouse tissues. Northern blot analysis of total RNA (20 μg) from mouse tissues was performed using pMD-9B (see Fig. 1, *Nco*I digest, 1.1 kb) as a probe. The positions of 28S and 18S ribosomal RNAs are indicated. Arrowheads indicate mRNAs of 2.8 kb and 1.5 kb, respectively. A, brain; B, thymus; C, lung; D, heart; E, liver; F, spleen; G, stomach; H, kidney; I, skeletal muscle. The bottom panel shows the ethidium bromide staining of a parallel gel to confirm that equivalent amounts of RNA were loaded on each lane.

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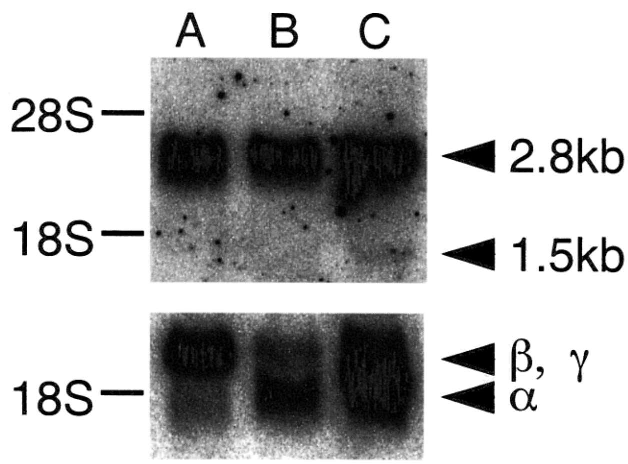

Fig. 4. Expression of α-Arp1 mRNA in cultured muscle cells as compared with that of actin. Upper: Northern blot analysis of total RNA (20 μg) from sol8 cells was performed using pMD-9B (*Nco*I digest, 1.1 kb) as a probe. The positions of 28S and 18S ribosomal RNAs are indicated. Arrowheads indicate mRNAs of 2.8 kb and 1.5 kb, respectively. A, Sol8 myoblasts in the growth medium; B, Sol8 myotubes cultured in the differentiation medium for 3 days; C, Sol8 myotubes cultured in the differentiation medium for 5 days. Bottom: the total RNA as in the upper panel was examined by Northern blotting using actin cDNA as a probe. Arrowheads indicate mRNAs of 2.8 kb and 1.5 kb, respectively. The position of 18S ribosomal RNA is also indicated. α, β and γ denote the mRNA of α-sarcomeric and β-, γ- cytoskeletal actins, respectively.

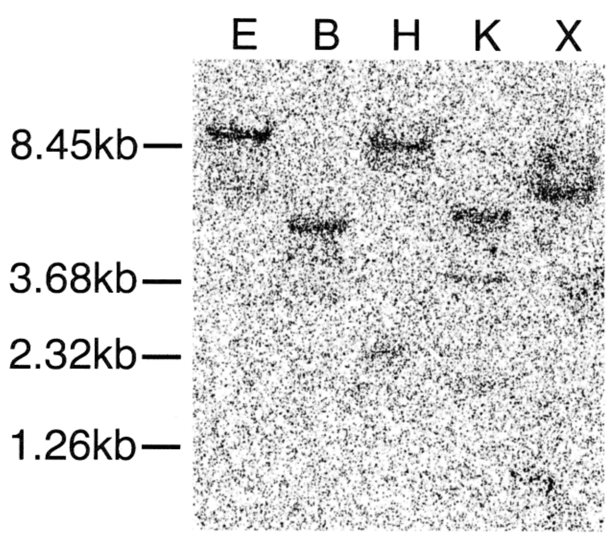

Fig. 5. Southern blot analysis of mouse genomic DNA. 10 μg of chromosomal DNA from mouse liver was digested with *Eco*RI (E), *Bam*HI (B), *Hin*dIII (H), *Kpn*I (K) and *Xba*I (X). Mobilities of size markers are shown in kilobase pairs (kb).

DISCUSSION

In this investigation, we determined the entire nucleotide sequence for mouse α-Arp1 and deduced the amino acid sequence. Since a wide variety of cell lines derived from mouse tissues is available, the cloned mouse α-Arp1 cDNA was thought to be useful for studying the functional roles of Arp1 in the cytoplasm at the molecular and cellular levels. Unexpectedly, we found that the amino acid sequence of mouse α-Arp1 was entirely the same as that of human α-Arp1, although the nucleotide sequences were slightly different; the identity in nucleotide sequence of the open reading frame between mouse and human was 90.5%. Interestingly, the peptide sequence is also identical with the *Drosophila* Arp1 sequence, while the identify in nucleotide sequence of the open reading frame was 90.0% (Fyrberg *et al.*, 1994). Thus, Arp1 seems to be a highly conserved protein among a variety of animals. Two variants of Arp1, α and β isoforms, have been described in human (Clark *et al.*, 1994). We were also able to isolate the cDNA encoding a protein with a sequence highly homologous with that of human β-Arp1 (Kusano and Obinata, unpublished data).

We cloned two variants of α-Arp1 cDNA from a mouse diaphragm cDNA library, which differ only by the inclusion or exclusion of 1,303 bp in the 3'-non-coding region but share a common open reading frame. It may be reasonable to assume that they were generated from a single gene by alternative RNA splicing. In agreement with this notion, a single gene for α-Arp1 was detected by Southern blot analysis of mouse genomic DNA. Two types of transcripts corresponding to large and small cDNAs, namely pMD-1C/pMD-7 and pMD-9B, were actually detected in muscle by Northern blotting. The longer type of α-Arp1 mRNA was transcribed most abundantly in brain among various mouse tissues, while the shorter one was found only in skeletal muscle. Clark *et al.* (1994) described that a small-sized transcript was detectable in heart, lung and skeletal muscle of human by using a cDNA probe for α-centractin (or α-Arp1). They called this γ-centractin, but as judged our present results, the γ-centractin seems to be identical with the small-sized α-Arp1 transcript which was derived from pMD-9B. A specific regulatory mechanism(s) for generating the smaller sized mRNA must exist in muscle cells. It has been reported that the 3'-non-coding region of cytoplasmic β- and γ-actin mRNAs is involved in the different intracellular localization between these isoactin mRNAs (Hill and Gunning, 1993). The α-Arp1 messages with or without the long insertion sequence in the 3'-non-coding region may differ in ability to localize along the intracellular structures.

While Arp1 was originally discovered as a component of the dynactin complex, a cytoplasmic dynein activator, recently the role of Arp1 on the actin cytoskeleton has come under closer scruting, since it has been described that Arp1 copolymerizes with conventional actin *in vitro* (Melki *et al.*, 1993). Considering that Arp1 is partly localized to centrosomes but mostly diffused in the cytoplasm of fibroblasts (Clark *et al.*, 1993), it is likely that Arp1 plays some important roles in

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the cytoplasm. However, so far nothing is known regarding the effects of Arp1 on the actin cytoskeleton in the cytoplasm. The present investigation showed that Arp1 is expressed significantly in muscle cells. Recently, it has been described that Arp1 could associate with CapZ in the dynactin complex (Schafer *et al.*, 1994), an F-actin capping protein which plays an important role at the early phase of myofibrillogenesis (Schafer *et al.*, 1995). It is then a matter of particular interest whether and how Arp1 is involved in actin filament organization in the process of myofibrillogenesis during muscle development, since the dynamic reorganization of actin filaments is especially dramatic during this process in muscle cells (Obinata, 1993).

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REFERENCES

Antin PB, Tokunaka S, Nachimas VT, Holtzer H (1986) Role of stress fiber-like structures in assembling nascent myofibrils in myosheets recovering from exposure to ethylmethanesulfonate. J Cell Biol 102: 1464–1479

Arx PV, Bantle S, Soldati T, Perriard J-C (1995) Dominant negative effect of cytoplasmic actin isoproteins on cardiomyocyte cytoarchitecture and function. J Cell Biol 131: 1759–1773

Blin N, Stafford DW (1976) A general method for isolation of high molecular weight DNA from eukaryotes. Nucleic Acids Res 3: 2303–2308

Chomczynski P, Sacchi N (1987) Single-step method of RNA isolation by acid guanidinium thiocyanate-phenol-chloroform extraction. Anal Biochem 162: 156–159

Clark SW, Meyer DI (1992) Centractin is an actin homologue associated with the centrosome. Nature 359: 246–250

Clark SW, Meyer DI (1993) Long lost cousins of actin. Curr Biol 3: 54–55

Clark SW, Staub O, Clark IB, Holzbaur ELF, Paschal BM, Vallee RB, Meyer DI (1994) β-Centractin: Characterization and distribution of a new member of the centractin family of actin-related proteins. Mol Biol Cell 5: 1301–1310

Feinberg AP, Vogelstein B (1983) A technique for radiolabeling DNA restriction endonuclease fragments to high specific activity. Anal Biochem 132: 6–13

Frankel S, Mooseker MS (1996) The actin-related proteins. Curr Opin Cell Biol 8: 30–37

Fyrberg C, Ryan L, Kenton M, Fyrberg E (1994) Genes encoding actin-related proteins of *Drosophila melanogaster*. J Mol Biol 241: 498–503

Gill SR, Schroer TA, Szilak I, Steuer ER, Sheetz MP, Cleveland DW (1991) Dynactin, a conserved, ubiquitously expressed component of an activator of vesicle motility mediated by cytoplasmic dynein. J Cell Biol 115: 1639–1650

Hayakawa K, Ono S, Nagaoka R, Saito O, Obinata T (1996) Differential assembly of cytoskeletal and sarcomeric actins in developing skeletal muscle cells *in vitro*. Zool Sci 13: 509–517

Herman IM (1993) Actin isoforms. Curr Opin Cell Biol 5: 48–55

Hill MA, Gunning P (1993) Beta and gamma actin mRNAs are differentially located within myoblasts. J Cell Biol 122: 825–832

Lees-Miller JP, Helfman DM, Schroer TA (1992) A vertebrate actin-related protein is a component of a multisubunit complex involved in microtubule-based vesicle motility. Nature 359: 244–246

Melki R, Vainberg IE, Chow RL, Cowan NJ (1993) Chaperonin-mediated folding of vertebrate actin-related protein and γ-tubulin. J Cell Biol 122: 1301–1310

Mulle C, Benoit P, Pinset C, Roa M, Changeux J-P (1988) Calcitonin gene-related peptide enhances the rate of desensitization of the nicotinic acetylcholine receptor in cultured mouse muscle cells. Proc Natl Acad Sci USA 85: 5728–5732

Mullins RD, Kelleher JF, Pollard TD (1996) Actin' like actin? Trends Cell Biol 6: 208–211

Obinata T (1993) Contractile proteins and myofibrillogenesis. Int Rev Cytol 143: 153–189

Rubenstein PA (1990) The functional importance of multiple actin isoforms. Bioessays 12: 309–315

Sambrook J, Fritsch EF, Maniatis T (1989) Molecular Cloning. A Laboratory Manual. Cold Spring Harbor Laboratory, Cold Spring Harbor, NY

Sanger F, Nicklen S, Coulson AR (1977) DNA sequencing with chain-terminating inhibitors. Proc Natl Acad Sci USA 74: 5463–5467

Schafer DA, Gill SR, Cooper JA, Heuser JE, Schroer TA (1994) Ultrastructural analysis of the dynactin complex: an actin-related protein is a component of a filament that resembles F-actin. J Cell Biol 126: 403–412

Schafer DA, Hug C, Cooper JA (1995) Inhibition of CapZ during myofibrillogenesis alters assembly of actin filaments. J Cell Biol 128: 61–70

Schroer TA (1994) New insights into the interaction of cytoplasmic dynein with the actin-related protein, Arp1. J Cell Biol 127:1–4

Schroer TA, Fyrberg E, Cooper JA, Waterston RH, Helfman D, Pollard TD, Meyer DI (1994) Actin-related protein nomenclature and classification. J Cell Biol 127: 1777–1778

Schroer TA, Bingham JB, Gill SR (1996) Actin-related protein 1 and cytoplasmic dynein-based motility—what's the connection? Trends Cell Biol 6: 212–215

Shimizu N, Obinata T (1986) Actin concentration and monomer-polymer ratio in developing chicken skeletal muscle. J Biochem 99: 751–759

Thomas PS (1980) Hybridization of denatured RNA and small DNA fragments transferred to nitrocellulose. Proc Natl Acad Sci USA 77: 5201–5205

Vandekerckhove J, Weber K (1978) At least six different actins are expressed in higher mammals: an analysis based on the amino acid sequence of the amino terminal tryptic peptides. J Mol Biol 126: 783–802

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